The Pharma Innovation Journal 2023; SP-12(9): 585-588

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(9): 585-588

www.thepharmajournal.com
Received: 18-06-2023
Accepted: 22-08-2023

Yogesh
Department of Veterinary Anatomy and Histology, International Institute of Veterinary Education and Research, Rohtak, Haryana, India

Santhosh Gaikwad
Department of Veterinary Anatomy and Histology, Mumbai Veterinary College, Mumbai, Parel, Mumbai, Maharashtra, India

Gururaj VK
Department of Veterinary Physiology, International Institute of Veterinary Education and Research, Rohtak, Haryana, India

Kalicharan Nayal
Department of Livestock Production and Management, International Institute of Veterinary Education and Research, Rohtak, Haryana, India

Corresponding Author:
Yogesh
Department of Veterinary Anatomy and Histology, International Institute of Veterinary Education and Research, Rohtak, Haryana, India

Cellular structure of the testes of indigenous dog (*Canis domesticus*) revealed using various special stain

Yogesh, Santhosh Gaikwad, Gururaj VK and Kalicharan Nayal

Abstract
The present research work was conducted on "Gross anatomical and histomorphological studies on testis of dog (Canis domesticus)". The testis of dogs collected from 6 non-descript, healthy adult dogs at sterilization center BSPCA, Parel, Mumbai. The normal structure of a dog's testis was investigated by using sections stained with hematoxylin and eosin, Van Gieson's stain for collagen fibres, Weigert's technique for elastic fibres and Wilders stain for reticular fibres. While periodic Acid Schiffs techniques for glycogen, Gomori's for alkaline phosphatase for alkaline phosphatase, and Gomori's modified lead Nitrate method for acid phosphatase were determined. PAS staining reaction revealed that the glycogen (mucopolysaccharides) was present in the various component of testis from intense to moderates. The alkaline phosphatase and acid phosphatase activity were also been observed from moderate to mild form in various component of testis.

Keywords: Canis domesticus, testis, wilders stain, Van Gieson's stain, Weigert's technique

1. Introduction
The dog, both domestic and wild, developed from "Miacis" in history. They are Canids or Canidae, which word Canine, comes from. Dogs are said to be descended from numerous variations of real wolves, including the possibility of Jackals and perhaps Foxes. Dogs were the first animal to be domesticated in several regions almost at the same time. Coyotes (*Canis latrans*), Azara dogs (Canis azrae), and other Canidae may have influenced the domestic dog. In agriculture, the dog was the first species to be domesticated by hunter. As a result of their long contact with humans, dogs have grown into a large number of domesticated individuals. Dogs have evolved to be specially attuned to human behavior over millennia, and the human-canine link has been the subject of much research. The dog has been selected for a variety of behaviors, sensory capacities, and morphological characteristics. Bhagyalakshmi *et al*. (2020) [5] studied the ten dog's testes. They reported that testis were compound tubular glands reddish-white in color with oval shape and located within the scrotum. Girish (1998) [15] conducted histological and histochemical studies on mediastinum testis and rete testis of the dog. He recorded that the epithelium of rete testis both intratesticular and extra testicular rested on PAS positive basal lamina, this epithelium showed mild reactions for lipids, AKPase and ACPase and succinic dehydrogenase enzymes. Anuradha *et al*. (2006) [1] conducted histomorphological and histochemical studies on the left and right testes of four adult Dalmantian dogs. The spermatogenic and leydig cells showed a moderate Alcian Blue and Periodic Acid Schiff (AB/PAS) reaction. The basement membrane of seminiferous tubules was strongly positive for neutral and acid mucopolysachharids.

2. Materials and Methods
For the present study, both the testis from non-descript healthy adult dog, whose body weight was ranging from 14 to 17 kg, was collected immediately after castration at BSPCA Hospital, Parel, Mumbai. The study was carried out at the Department of Anatomy and Histology, Mumbai Veterinary College, parel. The tissue pieces of 3 to 5 millimetre were cut from the various parts of testis and fixed in 10% neutral buffered formalin for 24 to 48 hours, then tissues pieces were treated with routine method of dehydration in ascending grades of alcohol, cleared in cedar wood oil and embedded in paraffin wax as per the method of Drury and Wallington (1967).

The paraffin block was sectioned at 3 to 5 µm thickness. The prepared slides were stained with following stains to make the histological observations (Singh and Sulochana, 1996-1997).

1. Mayer's Haematoxylin and Eosin for general histological observations.
2. Van Gieson's stain for collagen fibers.
3. Weigert's stain for elastic fibers.
4. Wilder's stain for reticular fibers.

3. Results and Discussion

Van Gieson's stain revealed collagen fibers at the capsule and around the basement membrane of the seminiferous tubule. Wilders stain revealed reticular fibers largely in capsule. Weigert's stain revealed elastic fibers largely in the blood vessels of the tunica Vasculosa (figure 1, 2, 3 and 4). The fragile loose connective tissue fibers and blood arteries that surrounded the seminiferous tubule made up the interstitial tissue. Sisson (1961) [24] reported that in dog's testis the mediastinum was centrally located and highly developed. It resulted in connective tissue septa, which split the testis into incomplete lobules. Frandson (1986) [11] mentioned that the number of fibrous septa, trabecular, passed inward from the tunica albuginia to form stroma that divided the gland into lobule. Each lobule was consisted of many seminiferous tubules. Mariano (1989) [21] stated that septa of connective tissue extending from the tunica albuginea, divided the testis into lobules partially or entirely. Seminiferous tubules Under microscope, these tubules appeared as an oval, elongated structure. Each tubule's wall was made up of connective tissue, with homogeneous basement membrane, and cuboidal epithelium. They consist of two types of cells, spermatogenic cells and Sertoli cells. These observations found accordance with the findings of William (1986) [3], Dellmann (1992) [8], Bacha and Linda (2007) [3], Gouletsou *et al.* (2010) [16], Trautman *et al.* (2015) [26], Aughey and Frye (2001) [2], Anuradha (2006) [1], Bhagyalakshmi *et al.* (2020) [5]. Spermatogenic cells These cells were arranged in four to eight layers from the basement membrane to the lumen. The differentiation of the basal cuboidal cells produced these cells. Four consecutive stages were identified, spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids. These observations found accordance with the finding of Banks (1986) [4], Mariano (1989) [21], Latshaw (1987) [19], Dellman (1992) [8], Girish (1998) [15], Bacha and Linda (2007) [3], Gouletsou *et al.* (2010) [16], Trautmann *et al.*(2015) [26], Aughey and Frye (2001) [2], Anuradha (2006) [1] and Bhagyalakshmi *et al.* (2020) [5]. Spermatogonia These cells form an outer layer of cuboidal cells with small nuclei on the basal lamina between sertoli cells. These were made up of primordial germ cells.

Periodic acid Schiff reaction stain for mucopolysacchride The glycogen of the testis was demonstrated using the periodic acid Schiff (PAS) staining method. PAS reaction was moderately positive in the capsule, spermatogonia, primary and secondary spermatocytes, sertoli cells, and Leydig cells, indicating the presence of mucopolysaccharides (Figure 8). PAS reaction was significantly positive in the basement membrane of seminiferous tubules, indicating the presence of mucopolysaccharides. These observations found in accordance with the observation reported by Bordoli and Dhingra (1983) [6], Bhagyalakshmi *et al.* (2020) [5], Girish (1998) [15] reported the distinct PAS activity at the basement membrane of epithelium of rete testis in dogs. PAS staining reaction revealed that the glycogen (mucopolysaccharides) was present in the various component of testis from intense to moderates. The alkaline phosphatase and acid phosphatase activity were also been observed from moderate to mild form in various component of testis.

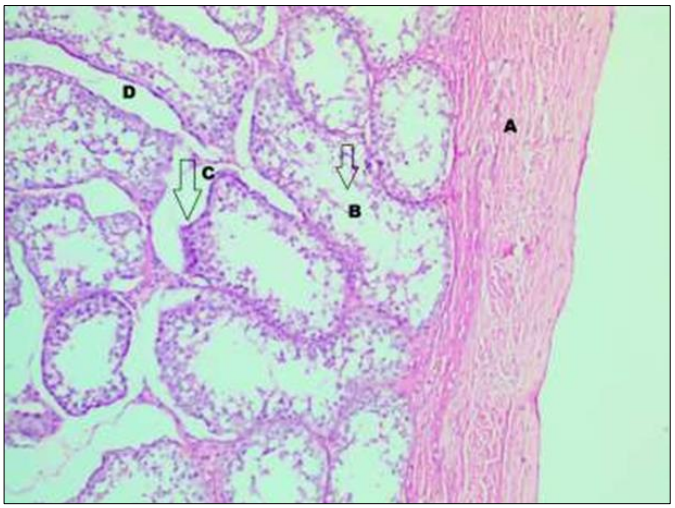

Fig 1: Photomicrograph of testis showing A- Testicular capsule Lumen of Seminiferous tubule (C) Seminiferous tubules (D) Interstitial space (H & E Stain, 10x)

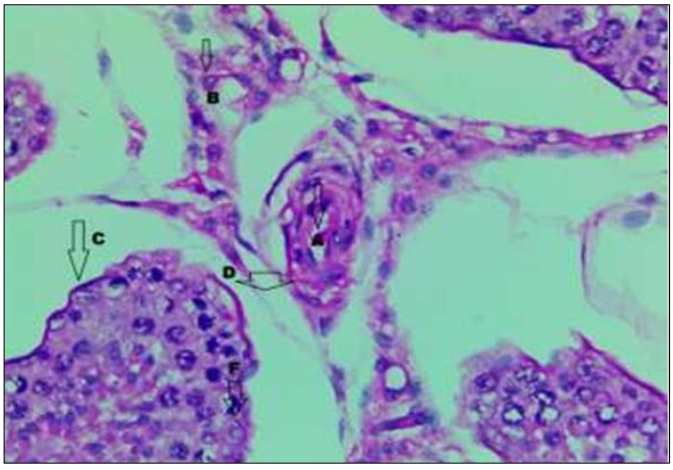

Fig 2: Microphotograph showing (A) Blood vessels, (B) Leydig cells, Seminiferous tubules, (D) Interstitial space, (F) Sertoli cells (Haematoxylin and eosin stain, 40x)

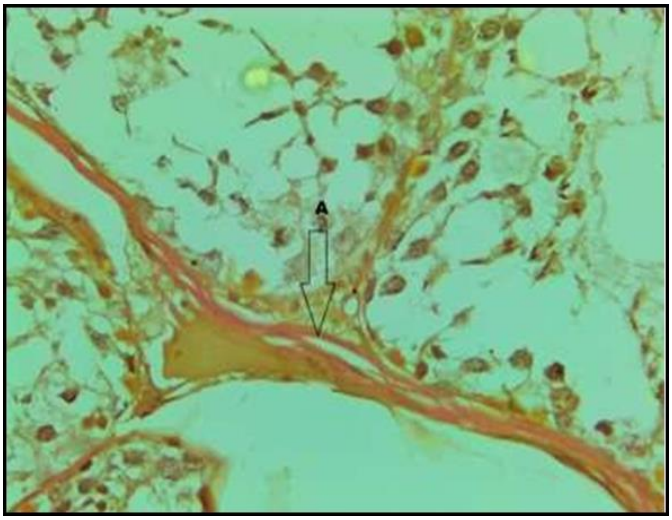

Fig 3: Microphotograph showing (A) Collagen fiber (Van Gieson stain, 40x)

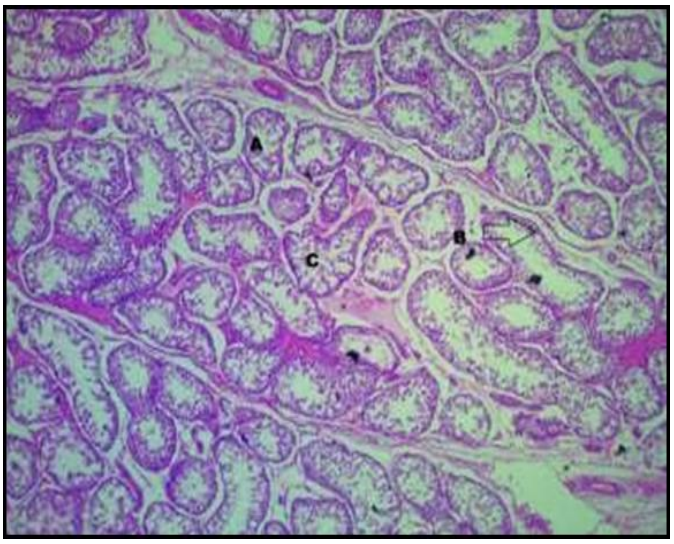

Fig 4: Microphotograph of testis showing (A) Seminiferous lumen (B) Trabeculae (C) Seminiferous tubules (H&E stain, 10X)

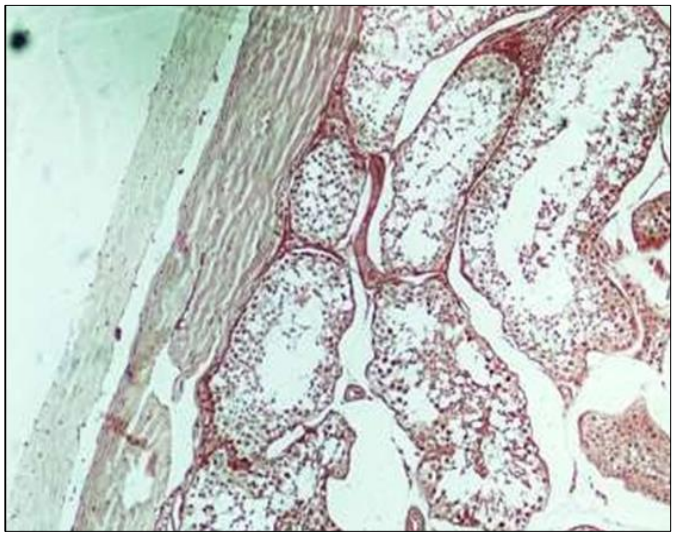

Fig 5: Microphotograph of testis showing black colored reticular fibers (Wilder's Stain, 10X

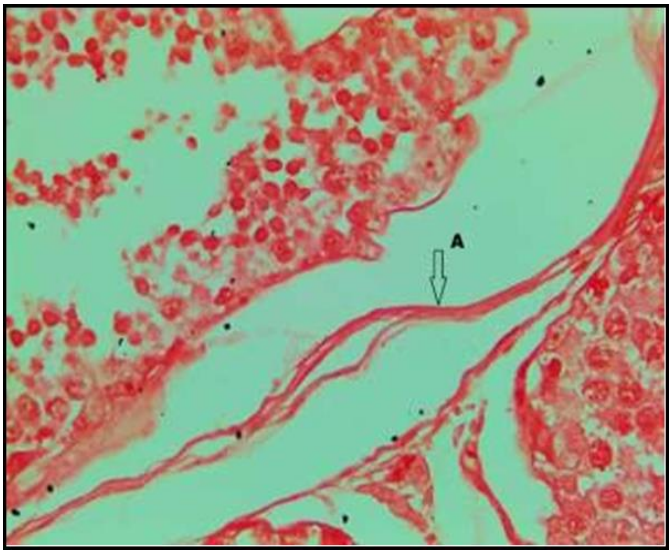

Fig 6: Microphotograph of testis Arrow showing bundle elastic fibers (Weigert stain, 40X)

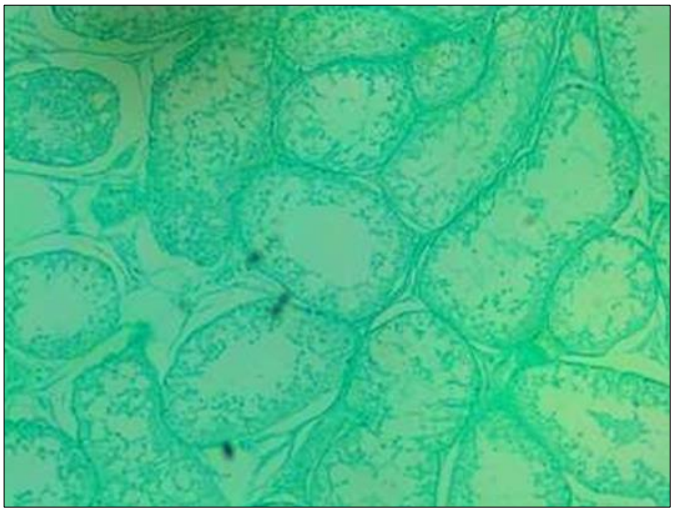

Fig 7: Microphotograph of testis showing acid phosphatase activity (Gomori's modified lead nitrate method, 10X

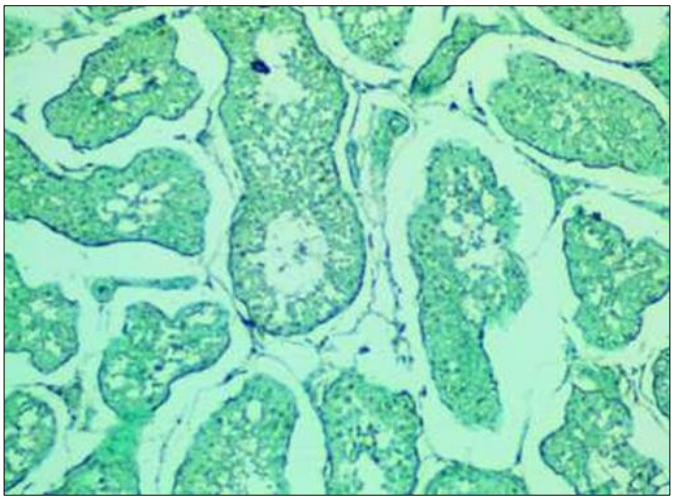

Fig 8: Microphotograph of testis showing alkaline phosphatase activity (Gomori's modified Alkline cobalt method, 10X)

4. Conclusion

1. Van Gieson's stain revealed collagen fibers at the capsule and around the basement membrane of the seminiferous tubule
2. PAS staining reaction revealed that the glycogen (mucopolysaccharides) was present in the various component of testis from intense to moderates.
3. The alkaline phosphatase and acid phosphatase activity were also been observed from moderate to mild form in various component of testis.
4. Wilders stain revealed reticular fibers largely in capsule
5. Weigert's stain revealed elastic fibers largely in the blood vessels of the tunica Vasculosa.

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